STUDIES ON THE METABOLISM OF MYCOBACTERIUM TUBERCULOSIS

III. The Growth of Mycobacterium Tuberculosis var. hominis in the Presence of Various Intermediates of the Dissimilation of Glucose to Pyruvic Acid

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The metabolic pathway by which mycobacteria break down glucose to pyruvic acid is even more obscure than the terminal metabolic process by which pyruvic acid is oxidized to carbon dioxide and water. Because of the strict aerobic nature to tubercle bacilli (Loebel, Shorr, and Richardson, 1933) it generally has been felt that anaerobic glycolysis could not begin unless, possibly, an initial aerobic reaction sparked the glycolysis (Edson, 1951).

Edson and Hunter (1943) found that gluconic acid, fructose-monophosphate, dihydroxyacetone, and glyceric acid were ineffectual for the stimulation of the growth of Mycobacterium phlei. Edson (1951) stated that Faine, Whiteside, and Edson, while investigating the possibility of an anaerobic glycolytic process functioning in the metabolism of mycobacteria, observed that intact cells of M. smegmatis and M. phlei did not respond greatly to the hexose phosphates, but that extracts of these organisms reduced methylene blue in vacuo in the presence of glucose-1-phosphate and fructose-1,6-phosphate. When methylene blue was absent, fructose-diphosphate was changed to a triosephosphate. A further observation indicated that the same extracts could convert phosphoglycerate to pyruvic acid and inorganic phosphate. Since these findings indicate the occurrence of the enzymes aldolase, triosephosphate dehydrogenase, and enolase in saprophytic acid-fast bacteria, Edson (1951) has suggested the possibility of an Embden-Meyerhof glycolytic scheme operating in the metabolism of these organisms.

The purpose of the present investigation was to determine whether certain substrates which may occur in the dissimilation of glucose to pyruvic acid, as given in the outline of Barron (1951), would support the subsurface growth of the highly virulent strain, H37Rv, of M. tuberculosis var. hominis. Theoretically, unless cell permeability is a limiting factor, an intermediate metabolic product should stimulate to a similar degree not only the rate of growth but also the growth of the same small numbers of organisms as the parent substrate.

METHODS

The small inocula method used to determine the rate of growth of the virulent H37Rv strain of M. tuberculosis and all other technical procedures were the same as described in the first paper of this series (Youmans and Youmans, 1953).

RESULTS

Listed in table 1 are the generation times, in hours, of the H37Rv strain when grown in the presence of various concentrations of many of the substrates which may be intermediate products in the metabolism of glucose. Growth of all five of the inocula occurred in the presence of certain concentrations of glucose, gluconic acid, pyruvic acid, lactic acid, glycerol, glyceraldehyde, and glyceraldehyde. With the latter two compounds, the mass of growth did not continue to increase appreciably during the five week period of incubation, and the generation time of the organisms in the medium containing glyceraldehyde was not reproducible.

In the medium containing fructose-6-phosphate, hexose-diphosphate, 3-phosphoglyceric acid, or α-glycerophosphate, growth appeared only in the cultures containing the 10⁻² mg inoculum. Actually, optimal growth was obtained only in the presence of glucose, gluconic acid, lactic acid, pyruvic acid, and glycerol. Lactic acid, pyruvic acid, and glycerol stimulated the rate of growth of strain H37Rv to a greater degree than did glucose or gluconic acid. Glucose and gluconic acid, however, in their optimal concentrations for...
supporting growth, stimulated the rate of growth to an approximately similar degree.

**DISCUSSION**

Although the anaerobic glycolytic scheme has been defined partially, the steps by which glucose is oxidized aerobically to pyruvic acid are relatively unknown. Generally it is believed that glucose may be oxidized aerobically also by way of the Embden-Meyerhof scheme (Dickens, 1951). However, there is some evidence to suggest (Dickens, 1938; Dickens and Glock, 1950) has indicated that the pentose is ribose. Cohen and Scott (1950) have confirmed these findings. Although none of the pentoses tested appeared to support the growth of the H37Rv strain (Youmans and Youmans, 1953), in the present study gluconic acid and glucose stimulated the rate of growth to an approximately similar degree. Furthermore, both compounds supported the growth of the smallest inoculum employed. This is in marked contrast to the results obtained in the

**TABLE 1**

The rate of growth of *Mycobacterium tuberculosis* var. *hominis* in the presence of various intermediates of the dissimilation of glucose to pyruvic acid

<table>
<thead>
<tr>
<th>COMPOUNDS</th>
<th>GENERATION TIME IN HOURS</th>
<th>CONCENTRATION OF COMPOUND IN PER CENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>24.0</td>
<td>0</td>
</tr>
<tr>
<td>Gluonic acid</td>
<td>24.0</td>
<td>0</td>
</tr>
<tr>
<td>Phosphogluconic acid</td>
<td>10^2*</td>
<td>26.2 (10^{-4}*</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hexose-diphosphate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glyceraldehyde</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3-Phosphoglyceric acid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glyceric acid</td>
<td>0</td>
<td>52.2</td>
</tr>
<tr>
<td>Pyruvic acid</td>
<td>0</td>
<td>20.5</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0</td>
<td>22.7</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α-Glycerophosphate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>21.7</td>
<td>24.0†</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

0 = no growth  
* = smallest inoculum (in milligrams) which grew  
† = slight growth.

an alternate mechanism by which glucose would be oxidized to gluconic acid, and then possibly by a series of decarboxylations via the α-keto acids to pyruvic acid (Dickens, 1951). In support of this, Campbell and Norris (1950) have obtained evidence that *Pseudomonas aeruginosa* oxidizes glucose not by way of the Embden-Meyerhof scheme but by the way of gluconic and 2-keto-gluconic acids. Dickens (1936) has obtained similar evidence for the oxidation of glucose in yeast. Moreover, he has suggested that ketogluconic acid is decarboxylated to a pentose. Further work in the presence of compounds which are considered to be involved in the glycolytic process which, on the whole, either did not support growth at all or only permitted growth of the largest inoculum, 10^{-4} mg used. With the exception of gluconic acid, these results are similar to those reported by Edson and Hunter (1943) with *M. phlei*.

The relative lack of response of the tubercle bacilli to the substances which may be involved in the Embden-Meyerhof scheme may be due to their inability to penetrate the bacterial cell. According to Edson (1951), Faine, Whiteside, and
Edson found that the oxygen uptake of intact cells of M. smegmatis and M. phlei was not stimulated greatly by the hexose phosphates although the oxygen uptake of cell-free extracts was stimulated. Thus, these organisms appeared to possess several enzymes of the glycolytic system. In this connection, it is interesting to point out that in our study while glucose, gluconic acid, glyceral, and glyceric acid stimulated the growth of the H37Rv strain and, therefore, were presumably absorbed into the cell the phosphorylated forms of these compounds did not permit appreciable growth. Whether these findings resulted from differences in cell permeability or from the inability of the bacterial cells to metabolize these phosphorylated compounds, is unknown. If, however, cell permeability to these substances is not a factor, the results would suggest an inefficiently operating glycolytic system.

It is also of interest that the substrates glyceral, lactate, and pyruvate, which would be among the final products of either the aerobic or the anaerobic metabolism of glucose, stimulated the growth of the H37Rv strain to a greater degree than did glucose itself. This finding might indicate that the terminal metabolic process involving the oxidation of these substrates to carbon dioxide and water may be carried out more efficiently by virulent tubercle bacilli than the early stages of glucose oxidation.

Respiratory studies using cell-free extracts of virulent mycobacteria might shed further light on the above problems.

Summary

The rate of growth of Mycobacterium tuberculosis var. hominis (strain H37Rv) in the presence of various intermediates of the dissimilation of glucose to pyruvate acid was investigated. It was found that gluconic acid and glucose stimulated the growth of the organisms to an approximately similar degree. In contrast, the various substrates examined which are involved in the glycolytic process did not support growth appreciably. Pyruvic acid and lactic acid, end products in the oxidation of glucose, stimulated the rate of growth to a greater degree than did glucose, suggesting perhaps the presence of a more efficient terminal metabolic process for this strain.

References


